

Imidacloprid mobility and longevity in soil columns at a termiticidal application rate

Chris J Peterson*

USDA Forest Service, Wood Products Insect Research Unit, Starkville, MS 39759, USA

Abstract: The mobility, longevity and termiticidal activity of imidacloprid (Premise® 2 termiticide; Bayer Environmental Sciences) at the termiticidal labeled rate for perimeter treatment were tested in vegetated and non-vegetated soil columns in two tests: in cone plots and in polyvinyl chloride (PVC) pipes. Imidacloprid content in the cone plot eluate peaked at 1 month, declined rapidly by the second month and then entered a lagging phase. The concentration of imidacloprid in the cone plot soil declined from $84.5 \mu\text{g g}^{-1}$ initially to $7.5 \mu\text{g g}^{-1}$ (non-vegetated plots) and $8.1 \mu\text{g g}^{-1}$ (vegetated plots) 6 months later. Neither eluate concentration nor soil concentration was affected by the presence of vegetation in the cone plots. In the PVC pipes, the top 15 cm of which was treated with Premise® 2 at the perimeter labeled rate, imidacloprid half-life was estimated at 6–9 months for vegetated and non-vegetated soil. Extractable imidacloprid declined more rapidly in the first 15 months than afterwards. Mobility of imidacloprid into lower, untreated soil depths was higher in non-vegetated pipes, and was likely due to the effect of vegetation on soil moisture. The presence of vegetation had little effect on the termiticidal activity of treated soil in the PVC pipes.

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1 INTRODUCTION

Imidacloprid is a leading insecticidal active ingredient in the United States, formulated for termite control as Premise® 75 WP, Premise® 2 and Premise® 0.5 by Bayer Crop Sciences (Research Triangle Park, NC, USA). It is also marketed overseas by other names (such as Termex 350 SC in India).

As a preconstruction preventative against termites (Isoptera), the imidacloprid 240 g L^{-1} SC, Premise® 2, is applied in a total application volume of 4 L m^{-2} ($1 \text{ gal } 10(\text{ft}^2)^{-1}$) to the soil before the concrete slab is poured (subslab pretreatment).¹ Imidacloprid is also used in post-construction perimeter treatments, where a trench is dug along the structure's foundation and the insecticide formulation is applied at $14 \text{ L } (3 \text{ m})^{-1}$ [$4 \text{ gal } (10 \text{ ft})^{-1}$] of trench for each 30 cm (1 ft) of trench depth (perimeter treatment).

The labeled rates applied for termite control are several times higher than those indicated for row crops. For example, when imidacloprid is applied according to the Premise® 2 label¹ for prevention of termites beneath a building slab, it is applied at $20.4 \text{ kg AI ha}^{-1}$ (18 lbs acre^{-1}), while Admire® PRO Systemic Protectant is applied to fruiting vegetables at a maximum rate of $0.575 \text{ kg AI ha}^{-1}$ ($0.50 \text{ lbs acre}^{-1}$).² Because termite control products are applied in and around living and working areas, their fate and longevity at the rates applied need to be examined directly.

Imidacloprid longevity in soil is affected by several processes occurring simultaneously, including

sorption to soil particles, degradation (biotic and abiotic), mobility and translocation into plants.³ In a laboratory study where no water was added to treated soil, imidacloprid had a half-life of 990–1230 days (about 32–40 months) at the termiticidal labeled rate.⁴ In another study, the half-life of imidacloprid was 156 days (about 5 months) in the laboratory and 74–174 days (1.5–5.75 months) in the field.³ In field studies simulating subslab treatments for termites there was a 70% reduction in imidacloprid concentration in the top 2.5 cm of soil 90 days after treatment.⁵ In a perimeter treatment field study, imidacloprid was detected at low levels or not at all 1 year after application near structures.⁶

Imidacloprid is soluble in water at 510 mg L^{-1} , more than other termiticide active ingredients, such as fipronil (2 mg L^{-1}), permethrin ($<1 \text{ mg L}^{-1}$), chlorpyrifos (2 mg L^{-1}) or chlordane (insoluble).⁷ Water solubility is an important property in evaluating the mobility of a compound. Mobility out of treated soil has the potential to shorten the effective life of the product and could result in environmental contamination. Studies are divided on the issue of imidacloprid mobility. Imidacloprid was highly mobile in a 25 cm sandy loam soil column, and 26% of the applied compound was collected in the eluate in a single washing.⁸ Similarly, 82% of applied technical-grade imidacloprid was recovered in the eluate of 20 cm soil columns, but the use of controlled-release formulations lessened mobility.⁹ In another study, however,

* Correspondence to: Chris J Peterson, USDA Forest Service, Wood Products Insect Research Unit, 201 Lincoln Green, Starkville, MS 39759, USA

E-mail: cjpeterson@fs.fed.us

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only trace amounts of imidacloprid were detected in the eluate of 65 cm columns, and only small amounts of imidacloprid percolated to 1 m below the surface in a lysimeter study.¹⁰ In reviewing these studies, it seems that imidacloprid was less mobile in soils that received periodic treatments of water^{10–12} than in studies where all the water was applied in one single flush.^{8,9,13} Few mobility studies have been published with imidacloprid formulated for termite control.^{5,13} There was a significant increase in imidacloprid residues in soil 2.6–7.6 cm below the soil surface 90 days after field application.⁵ Imidacloprid moved with the water front in an ascending soil column, although the concentration was highest near the origin.¹³

Landscape plants, turf grasses, ornamental shrubs, trees, bulbs, etc., are often planted in or near the treated barrier of a structure. The presence of vegetation was found significantly to lower the soil concentration of pesticides (such as atrazine)^{14,15} and environmental contaminants¹⁶ through both plant uptake and enhanced degradation. Imidacloprid is translocated from the soil into plants, and systemic activity is well documented. Soil applications of imidacloprid resulted in mortality to the Colorado potato beetle,¹⁷ cotton whitefly,¹⁸ citrus leaf miner,¹⁹ green peach aphid²⁰ and the beneficial coccinellid predator *Coleomegilla maculata* DeGeer.²¹ Radiolabeled imidacloprid applied as a seed dressing was detected in the leaves of sugar beet up to 97 days after planting.²² Imidacloprid was taken up continually, as rice plants transplanted out of treated soil showed a rapid decline in imidacloprid levels (half-life in the plant = 3 days).²³ Enhanced pesticide degradation, when observed, is often attributed to microorganisms associated with the root zone (rhizosphere) of plants, and rhizosphere bacteria are known to contain xenobiotic-metabolizing enzymes.²⁴ No published studies have directly examined the effect of vegetation on imidacloprid mobility and longevity at termiticidal application rates, although reductions in half-life owing to vegetation of 50–91 days³ or 142 days²⁵ have been observed at lower rates.

This study reports the mobility, longevity and termiticidal activity of imidacloprid in vegetated and non-vegetated soil columns in a greenhouse.

2 MATERIALS AND METHODS

2.1 Chemicals

Imidacloprid 240 g L⁻¹ SC (Premise® 2, lot 2 652 007, manufactured March–April 2002) was purchased from a commercial retailer (Pest Control Depot, Palm Bay, FL, USA). All organic solvents (methanol, acetonitrile) were Certified or Optima grade, purchased from Fisher Scientific (Hampton, NH, USA). Deionized water was obtained from a Barnstead Nanopure ultrapure water system (Dubuque, IA, USA).

2.2 Cone plot test

Soil was treated by placing 340 mL of soil (sandy loam, 15% silt, 75% sand, 10% clay, pH 7.8, 1.48%

organic matter) into 500 mL plastic jars in April 2003. Premise® 2 was diluted with water at the label rate of 2 mL L⁻¹ (480 mg AIL⁻¹), and 34 mL of this dilution, or distilled water for control groups, was added in three portions to each jar with vigorous shaking by hand after each portion was added. This corresponds to a soil moisture of 10%, and this soil becomes saturated at about 20–22% soil moisture (see discussion of PVC pipes, Section 3.2). There were three replications, and a different soil portion was treated for each replication. The jars sat for 2 h to allow the liquid to wick through the soil. The cone plots consisted of 21.5 cm tall × 4 cm inside diameter (top) and 2.4 cm inside diameter (bottom) UV-stabilized Ray Leach Cone-Tainers™ purchased from Hummert International (Earth City, MO, USA). The bottom of each cone was fitted with glass wool to prevent loss of soil, and treated or untreated soil was added to a depth of 15 cm. Unused soil was stored at –20 °C until extracted for imidacloprid content as described later in this section. Vegetated cones received Bermuda grass seed [*Cynodon dactylon* (L.) Pers.] (0.1 g), and the top 1 cm of soil was agitated to work in the seeds. The study consisted of four treatments: untreated soil with no vegetation, treated soil with no vegetation, untreated soil with vegetation and treated soil with vegetation.

Each cone was watered with 25 mL distilled water, and then another 20 mL was added to each cone and the eluate was collected. Water (30 mL, equivalent to an approximately 2.5 cm rainfall event) was added to each cone once per week, and once per month for 6 months each cone received enough water so that >10 but <15 mL eluate collected in a beaker. Eluate was cleaned up by using solid-phase extraction (SPE) cartridges (Accubond II ODS-C18; Agilent Technologies, Santa Clara, CA, USA) as follows: eluate (10 mL) was passed through one SPE cartridge, which was allowed to dry by pulling a vacuum through it for 15 min. Once dry, 1 mL of methanol was passed through the cartridge to elute the imidacloprid. The collected methanol was stored at –20 °C until analyzed by high-performance liquid chromatography (HPLC) for imidacloprid content. Analyses of cone plot eluate followed a previously described method²⁶ using a Waters Alliance 2695 liquid chromatograph (Waters Corp., Milford, MA, USA), with 10 µL injection, isocratic elution in acetonitrile + water (35 + 65 by volume) and 1 mL min⁻¹ flowrate on an ODS (C-18) column (4.6 × 75 mm) with UV detection (270 nm) on a Waters 996 photodiode array detector.

After 6 months, the cone plots were dismantled and all plant material was removed from the soil. The recovered soil was extracted by placing soil (20 ± 0.5 g) into a jar and adding acetonitrile + water (80 + 20 by volume, 40 mL) and shaking (200 rpm for 4 h). The jars settled for >72 h. The supernatant was decanted and vacuum filtered through glass fiber filters. The soil samples that were reserved and frozen at the time of treatment were also extracted at this time,

by using the same method. Cone plot soil extracts were analyzed by HPLC according to the method described for the PVC pipe soil described in Section 2.3. The extracts were stored at -20°C until analyzed by HPLC. The eluate data were analyzed by mixed analysis of variance on SAS²⁷ (SAS Institute, Cary, NC, USA) for repeated measures.

2.3 PVC pipe test

The longevity and mobility of imidacloprid were determined in 60×10 cm internal diameter polyvinyl chloride (PVC) pipes. Five 1 cm diameter drainage holes were cut into an 11.5 cm square plastic plate, and a piece of aluminum window screen was cut to the same size. The screen was sandwiched between the plastic plate and the bottom of the pipe and held together with adhesive. Greenhouse soil, as described in Section 2.2, was cleared of stones and large clumps by using a Royer conveyor (Royer Foundry and Machine Co., Kingston, PA, USA), which excluded particles greater than about 0.5 cm diameter. The pipes were filled with untreated soil (45 cm) and the soil was saturated with water to settle it and minimize later compaction. More soil was added to bring up to the 45 cm mark. The columns were allowed to drain for at least 48 h before the addition of the treated soil. In April 2003, additional soil was treated with Premise[®] 2 diluted at the label rate of 2 mL L^{-1} in water (480 mg AIL^{-1}) in a cement mixer by adding 3.8 L of diluted formulation to 34 L of soil. This is slightly above the perimeter treatment label rate of 4 US gallons (14.1 L) per 10 linear feet (3 m) per foot (30 cm) of depth in a trench 6 inches (15 cm) wide.¹ The diluted formulation was mixed and applied to the soil separately for each replication. Treated soil (15 cm) was added on top of the 45 cm of untreated soil. There were four treatments (treated/vegetated, untreated/vegetated, treated/non-vegetated, untreated/non-vegetated) in three replications. Vegetated plots were seeded with Bermuda grass seed (0.5 g), and the top 1 cm was agitated to cover seeds. The pipes were watered once per week ($200 \pm 10 \text{ mL}$) to simulate a 2.5 cm rainfall event. At the start of the test and at 3 month intervals for 2.5 years, the pipes for that time point were cut with a circular miter saw into eight 7.5 cm depths, and each depth was analyzed for moisture content, imidacloprid content and termiticidal activity. Soil moisture from each depth was determined by drying soil ($10 \pm 0.5 \text{ g}$) in an oven overnight (100°C), and percentage moisture was calculated by the change in mass.

Soil from each pipe depth was mixed for uniformity, and one sample ($10 \pm 0.5 \text{ g}$) per replication and soil depth was extracted with acetonitrile + water (80 + 20 by volume, 20 mL) by shaking (200 rpm for 4 h). The extract settled for $>72 \text{ h}$, and the supernatant was filtered through glass fiber filters.⁴ Extracts were analyzed by using HPLC with $20 \mu\text{L}$ injection, water + acetonitrile (60 + 40 by volume) mobile phase at

1 mL min^{-1} through a Whatman Partisphere RTF C-18 column ($4.6 \times 250 \text{ mm}$) (Whatman, Florham Park, NJ, USA) fitted with an Agilent XDB C-18 ($4.6 \times 12.5 \text{ mm}$) guard column (Santa Clara, CA, USA) and UV detection (270 nm). Extractable imidacloprid was calculated on a soil dry weight basis. This method produces 89.8–99.9% recovery in sandy loam soils at 100 mg kg^{-1} (data not shown). A split-plot arrangement in a completely randomized design was used, with each pipe (combination of vegetated state, time and treatment) as the whole plot factor, and soil depth as the subplot factor. Mixed analysis of variance on SAS²⁷ was used to determine significant effects on extractable imidacloprid by soil depth, time and vegetation.

2.4 Termite bioassay

A preliminary toxicity test was conducted to determine the range of imidacloprid concentrations in soil lethal to termites. One population of *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) was collected from Webster County, MS, in the spring of 2003. Greenhouse soil was treated with Premise[®] 2 to constitute 100, 10, 1, 0.1 and 0.01% of the labeled rate (69, 6.9, 0.69, 0.07 and $0.01 \mu\text{g g}^{-1}$ soil respectively), plus a control consisting of water, by applying appropriately diluted formulation (4 mL) to soil (36 g) and placing on a jar roller ($6 \pm 1 \text{ min}$). The diluted formulation was applied to each of the three replications separately. From each jar, soil was placed into three 60 mm diameter petri dishes for destructive sampling at 3, 7 and 14 days. Distilled water (1 mL) was added to each dish to maintain humidity, and each dish received a piece of cardboard (1 cm^2) as a food source. The additional 1 mL water plus the water in the termiticide application volume resulted in the soil being at 19% moisture. Worker termites (25) were added to each dish, and the number of surviving termites was counted at 3, 7 and 14 days. The test was run in an incubator at 25°C and 75% RH in the dark.

Soil recovered from pipes and cone plots was tested in a similar manner. A single petri dish was filled with soil (15 g) from each pipe depth or cone plot, and each dish was supplied with a 1 cm square of cardboard. Distilled water was added if the soil was dry; 1 mL if between 10 and 18% soil moisture and 2 mL if less than 10% soil moisture. Therefore, all bioassays were conducted at 17–23% soil moisture. This moisture level was necessary to prevent desiccation of the termites during the 14 day test. Termites (25 workers for cone plots or ten workers for PVC pipes) from a single population of *R. flavipes* were introduced into each dish at each time point, and the number of surviving termites were recorded non-destructively at 3, 7 and 14 days. The test was run in an incubator at 25°C and 75% RH in the dark. Percentage survival was converted to percentage mortality, and percentage mortality was corrected for control mortality by using Abbott's equation.²⁸ Corrected percentage mortality was transformed by taking the arcsin of the square

root before statistical analysis by using mixed analysis of variance on SAS.²⁷

3 RESULTS AND DISCUSSION

3.1 Preliminary toxicity test

To this population of termites, Premise® 2 was toxic within 7 days at 100 and 10% of the labeled rate (69 and $6.9 \mu\text{g g}^{-1}$) (Table 1). Premise was not toxic at rates of 1% of the labeled rate ($0.69 \mu\text{g g}^{-1}$) and lower within 14 days. The greatest reduction in survival was observed between 3 and 7 days, and no further reduction in survival beyond background mortality was observed between 7 and 14 days. A similar study²⁹ reported 1.5% mortality after 7 days of continuous exposure at $5 \mu\text{g g}^{-1}$ imidacloprid in sandy loam soil, 4.7% at $10 \mu\text{g g}^{-1}$ and 29.5% at $25 \mu\text{g g}^{-1}$, whereas in the present study $7.5\text{--}8.1 \mu\text{g g}^{-1}$ caused 100% mortality within 7 days (see Section 3.2). Imidacloprid activity differs in different soil types,²⁹ and, although the present soil is also a sandy loam, differences in organic matter, termite colony and test conditions may be responsible for differences between previous studies and the present study.

3.2 Cone plot test

Among the treated cone plots, time was the only factor that significantly affected imidacloprid concentration in the eluate ($F = 82.19$; $\text{df} = 6, 22$; $P < 0.0001$), although there was no decrease in eluate concentration at 3 months and beyond. For the vegetated plots, the eluate initially (time 0) contained 33.3 mg L^{-1} imidacloprid, peaked at 38.2 mg L^{-1} at 1 month and then declined to 6.1, 1.6, 0.8, 0.8 and 0.6 mg L^{-1} for months 2–6 respectively (Fig. 1). For non-vegetated plots, the eluate initially (time 0) contained 23.5 mg L^{-1} imidacloprid, peaked at 35.7 mg L^{-1} at 1 month and then declined to 12.6, 6.0, 3.0, 2.6 and 1.4 mg L^{-1} for months 2–6 respectively (Fig. 1). The effect of vegetation on eluate concentration was not statistically significant ($F = 0.08$; $\text{df} = 1, 2$; $P = 0.7994$).

The presence of imidacloprid in the eluate demonstrated that the termiticide moved with soil pore water. Most of the imidacloprid that moved did so in the first 2–3 months, and it is likely that the applied concentration was above the soil's holding capacity. Therefore, the amount of compound that washed off in the first

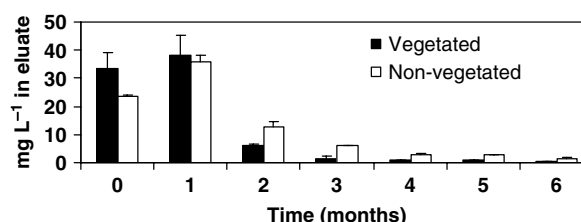


Figure 1. Imidacloprid concentration (mg L^{-1}) in eluate from vegetated and non-vegetated cone plots.

2 months represented the excess. Also, the amount of imidacloprid available to move in the soil is potentially reduced over time by mobility out of the plot, degradation and soil sorption.

During 6 months of weekly watering (25 washings of 30 mL; 62.5 cm total rainfall, 750 mL or $4\times$ the soil volume), the extractable soil concentration of imidacloprid declined from 84.5 to $7.5 \mu\text{g g}^{-1}$ (in non-vegetated plots) and $8.1 \mu\text{g g}^{-1}$ (vegetated plots). Soil aging, vegetation and weekly watering had inconsistent effects on the termiticidal activity of treated soil from cones. Initially, an average of 98.5% of termites died within 7 days in the treated soil. Six months later, 100% of the termites died within 7 days in soil recovered from the non-vegetated plots, and 67% of termites died within 7 days in soil recovered from the vegetated plots. This 67% represents two replications where 100% of the termites died and one replication where 0% of the termites died. Mortality in soil from the untreated plots was near 0%.

3.3 PVC pipe test

3.3.1 Soil moisture

The presence of vegetation had a significant effect on soil moisture (Fig. 2). Taking all time points and soil depths together, the effect of vegetation on soil moisture depended upon depth and time (i.e. there was a significant three-way interaction between depth, time and vegetation: $F = 2.50$; $\text{df} = 56, 252$; $P < 0.0001$). Moisture in the non-vegetated pipes stayed constant throughout the study within any given depth (except the 0–7.5 cm depth) and generally increased with increasing depth. Soil at the 45–52.5 cm and the 52.5–60 cm depths was always above 20% moisture (nearly saturated). In the vegetated pipes, which were almost without exception drier than non-vegetated pipes, soil moisture varied over the course of the study, and the driest soils were observed at 3, 18 and 27 months, which corresponded to July 2003, October 2004 and July 2005 respectively. Except for 0 months, when the seeds had not yet sprouted, the vegetated pipes were the moistest at 9 and 21 months, corresponding to January 2004 and January 2005 respectively. Vegetated pipes were driest during the periods of most vigorous plant growth and wettest during periods of relative plant quiescence. Because the soil moisture in non-vegetated pipes did not vary seasonally, temperature was not a factor in determining soil moisture. Grass roots were qualitatively

Table 1. Mortality of termites exposed to imidacloprid-treated soil

Dose ($\mu\text{g g}^{-1}$ soil)	Mortality (%) (\pm SEM)		
	3 days	7 days	14 days
69	50 (± 21)	100 (± 0)	100 (± 0)
6.9	30 (± 25)	80 (± 20)	87 (± 13)
0.69	43 (± 24)	6 (± 3)	17 (± 3)
0.07	37 (± 27)	12 (± 7)	17 (± 7)
0.01	17 (± 7)	7 (± 7)	23 (± 3)
Control	7 (± 7)	13 (± 7)	33 (± 3)

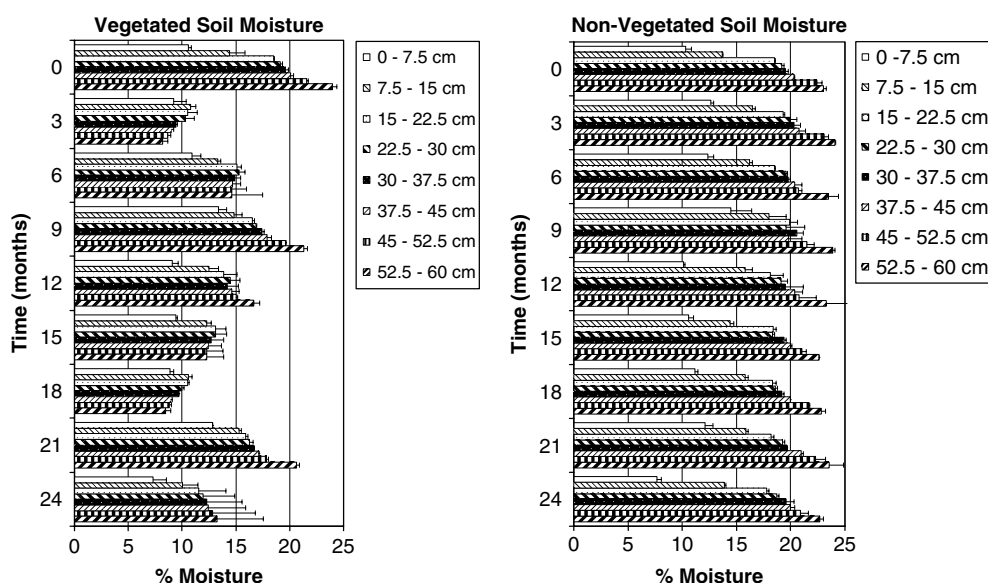


Figure 2. Soil moisture for vegetated and non-vegetated pipes at each depth and sampling time over the course of the study.

observed in all soil depths by 3 months, although the 0–7.5 cm depth contained most of the root mass.

3.3.2 Extractable imidacloprid

Extractable imidacloprid residues in the top two (treated) depths, 0–7.5 cm and 7.5–15 cm, of the PVC pipes declined in two phases: one phase from 0 to 15 months, and a second from 18 to 30 months (Fig. 3). Because of unequal variances in the two dissipation phases, statistical analyses were conducted

separately for 0–15 months and 18–24 months. It is possible that irreversible sorption of imidacloprid to soil particles and to the plastic pipes may have reduced the amount of imidacloprid extracted and the bioavailability to termites. Mass balance of imidacloprid was not conducted in this study owing to the difficulty of extracting imidacloprid from plant material, the diversity of imidacloprid metabolites, the inability to develop a satisfactory analysis method for all known imidacloprid metabolites, the inability to use

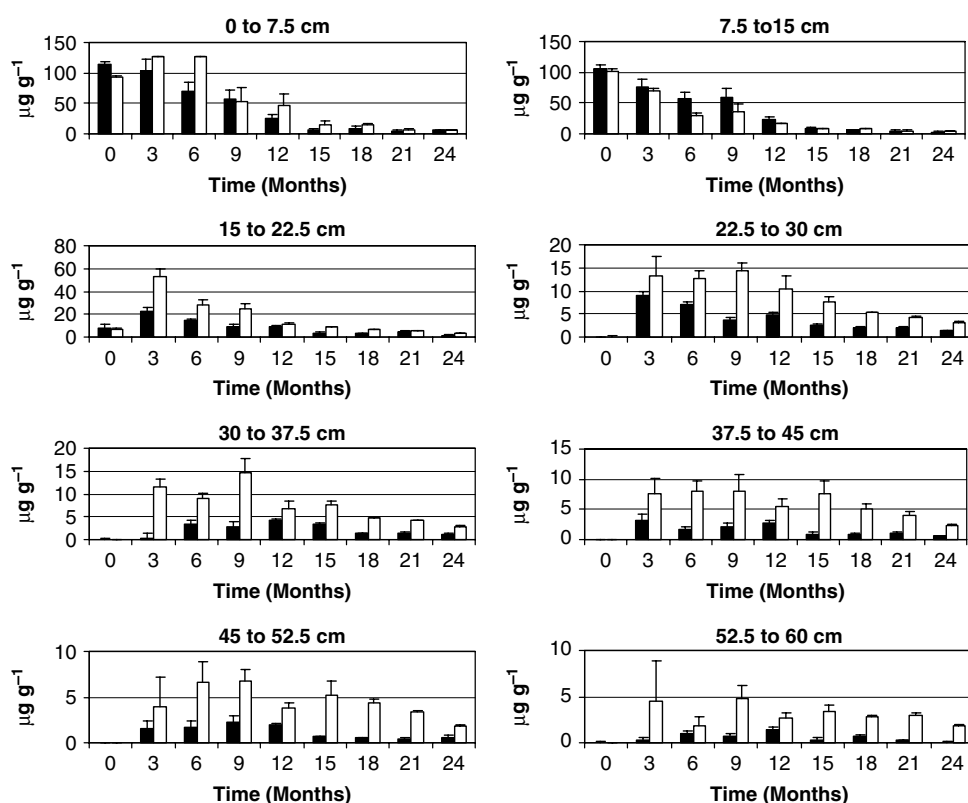


Figure 3. Extractable imidacloprid (mg L^{-1}) from vegetated pipes (solid bars) and non-vegetated pipes (open bars) for each soil depth. Note the change in scale in y-axis values.

radiolabeled imidacloprid and the observation that a majority of imidacloprid applied to soil is mineralized to carbon dioxide.¹¹ Data for pipes at 27 and 30 months were not included in the statistical analysis and are not shown in Fig. 3 owing to destruction of several of the vegetated pipes by fire ants earlier in the study. It is noteworthy that Premise® 2 is not labeled for fire ant control.

For the 0–7.5 cm and 7.5–15 cm depths, in the first 15 months there was a significant three-way interaction between depth, time and vegetation ($F = 3.27$; $df = 5, 20$; $P = 0.0256$), i.e. the effect of vegetation depended upon the combination of depth and time. Extractable imidacloprid was higher in the top depth (0–7.5 cm) of non-vegetated pipes than in the top depth of vegetated pipes at 3, 6 and 12 months, but was nearly equal at 9 and 15 months (Fig. 3). Extractable imidacloprid at the second depth (7.5–15 cm) was higher in vegetated than non-vegetated pipes at 6 and 9 months, but was nearly equal at 3, 12 and 15 months. When a difference in extractable imidacloprid was observed between vegetated and non-vegetated pipes, extractable imidacloprid was higher in non-vegetated pipes at the top depth but higher in vegetated pipes at the second depth. Although there was a numerical decrease in extractable imidacloprid in both treated depths, 0–7.5 cm and 7.5–15 cm, at 18 months and beyond there were no significant effects due to depth, time or vegetation, nor were there any significant interaction terms.

A half-life of extractable imidacloprid may be estimated at about 9 months at the 0–7.5 cm depth of non-vegetated pipes and 6 months in vegetated pipes, and around 6 months in both vegetated and non-vegetated pipes at the 7.5–15 cm depth (Fig. 3). This is much less than that reported in another study (990–1230 days, or about 32–40 months) where no water was added to the treated soil,⁴ but larger than other published values (74–174 days, or 2.4–5.7 months) where the soil received water.³

Vegetation reduced the half-life of imidacloprid by about 90 days at the 0–7.5 cm depth (Fig. 3). Similar studies found a reduction of about 50–91 days³ or 142 days²⁵ on account of vegetation. One study³ suggests that the reduction in half-life in cropped soils is due to microbial action associated with the vegetation, and in the present study the 0–7.5 cm depth contained most of the root mass of the vegetation (the root mass was not measured, and fine roots were observed at all depths by 3 months). Uptake of imidacloprid into vegetation would have been highest at this depth owing to the larger root mass, and any root-associated microorganisms capable of metabolizing imidacloprid would have been more plentiful at this depth.

Extractable imidacloprid at untreated depths (15–60 cm) in the first 15 months was analyzed separately from 18 to 24 months owing to unequal variances. For the first 15 months there was a significant three-way interaction between depth, time

and vegetation ($F = 3.54$; $df = 25, 120$; $P < 0.0001$), i.e. the effect of vegetation on extractable imidacloprid depended upon depth and time. No imidacloprid was detected below 22.5 cm initially (0 months), but imidacloprid was measured at all soil depths at 3 months and beyond (Fig. 3). At 18 months and beyond there were significant interactions between time and vegetation ($F = 12.37$; $df = 2, 12$; $P = 0.0012$) and time and depth ($F = 2.18$; $df = 10, 60$; $P = 0.0311$), i.e. the effects of both vegetation and depth on extractable imidacloprid depended upon time. From 3 months to the end of the study (30 months), extractable imidacloprid was always higher in untreated depths of non-vegetated pipes than in corresponding depths of vegetated pipes. Within any chosen time, extractable imidacloprid declined with greater depth; the 15–22.5 cm depth always had the highest extractable imidacloprid and the 52.5–60 cm depth nearly always had the lowest. With an increase in time, the effects of both vegetation and depth declined, and extractable imidacloprid at each depth converged (to about $1 \mu\text{g g}^{-1}$ in vegetated pipes and $2.5 \mu\text{g g}^{-1}$ in non-vegetated pipes).

Extractable imidacloprid at the 15–22.5 cm depth was in many cases 2–3 times higher than at the 22.5–30 cm depth. This is expected for two reasons. Firstly, the 15–22.5 cm depth is the first depth below the treated depths (0–7.5 and 7.5–15 cm) and is the direct recipient of water percolating through the treated soil. Secondly, some soil settling is unavoidable, with treated soil physically moving into the 15–22.5 cm depth. Also, when the pipes were cut, it is possible that the line of the cut was not exactly at the margin of the two soil depths, thus including treated soil from the 7.5–15 cm depth in the 15–22.5 cm depth. The effect of vegetation on soil moisture is likely responsible for the differences observed between vegetated and non-vegetated pipes. At the 7.5–15 cm depth, extractable imidacloprid was usually higher in vegetated pipes, possibly because the lower soil moisture decreased the amount of water, and therefore imidacloprid, moving into lower soil depths. Imidacloprid moved into untreated depths (15–60 cm) more readily in non-vegetated pipes. Because vegetated pipes were generally drier than non-vegetated pipes, water added at weekly intervals (200 mL) to vegetated pipes would not have penetrated as deeply, limiting the depth to which imidacloprid was able to move. Extractable imidacloprid was higher in non-vegetated pipes than in vegetated pipes at all time points (except 0 months) and at all soil depths. This is consistent with the observation noted in the Introduction that imidacloprid is less mobile when the soil has a chance to dry between watering (compare references 10–12 with references 8, 9 and 13).

3.3.3 Termite mortality

Corrected mortality data from the bioassays are presented in Fig. 4. In cases where fewer termites died in the treated dishes than in the control, Abbott's

equation is less than zero; such values were set equal to zero for the statistical analysis. In cases where all termites die in the control, Abbott's equation is undefined (the denominator is equal to 0) and the uncorrected mortality data were used. Data for pipes at 27 and 30 months were not included in the statistical analysis owing to destruction of several of the vegetated pipes by fire ants earlier in the study.

At the top two depths (i.e. treated soil) there was a significant interaction between the effects of time and vegetation on mortality ($F = 2.28$; $df = 8, 61$; $P = 0.0330$), i.e. the effect of vegetation on mortality depended upon time. At the 0–7.5 cm depth, mortality was equal to 100% in both vegetated and non-vegetated pipes from 0 to 6 months (Fig. 4). From 9 to 15 months, soil from vegetated pipes was more toxic to termites than soil from non-vegetated pipes. Mortality in soil from the 0–7.5 cm depth of non-vegetated pipes declined steadily from 100% at 6 months to 0% at 15 months, and was not consistently high afterwards. By 18 months, termite mortality from soil in the 0–7.5 cm depth of vegetated pipes had declined to 0%. At the 7.5–15 cm depth, both vegetated and non-vegetated soil caused 100% mortality at 0 months. Vegetated soil was less toxic than non-vegetated soil at 3 and 6 months, but was more toxic from 9 to 18 months. Soil from the 7.5–15 cm depth of non-vegetated pipes had low mortality starting at 12 months, and it remained low throughout the remainder of the study. For the treated depths, the

same trend is observed in the decline of extractable imidacloprid and the decline in termiticidal activity of the soil. By 18 months, extractable imidacloprid at the 0–7.5 cm depth had declined to 15 and 7% of initial (0 month) values for vegetated and non-vegetated pipes respectively, and for the 7.5–15 cm depth extractable imidacloprid had declined to 3 and 5% of initial (0 month) values for vegetated and non-vegetated pipes respectively. In a field study conducted near Gulfport, MS, from December 2003 to December 2005, soil treated with the labeled rate of Premise[®] 75 for perimeter and subslab pretreatments was not termiticidally active 1 year after application (data not shown). Also, penetration of imidacloprid-treated test plots by termites at this same site at the subslab pretreatment rate was observed 2 years after application.³⁰

In untreated depths (15–60 cm) there was a significant three-way interaction between the effects of vegetation, time and depth on mortality ($F = 1.49$; $df = 40, 208$; $P = 0.0394$), i.e. the effect of vegetation on mortality depended upon depth and time. In general, mortality at all times decreased with increasing depth, and mortality at all depths decreased with increasing time. Soil from the 15–22.5 cm depth was generally the most active of the untreated depths (producing >90% mortality in non-vegetated pipes at 3, 6 and 9 months), and soil from non-vegetated pipes was generally more active than soil from vegetated pipes. Dose–response relationships were generally true

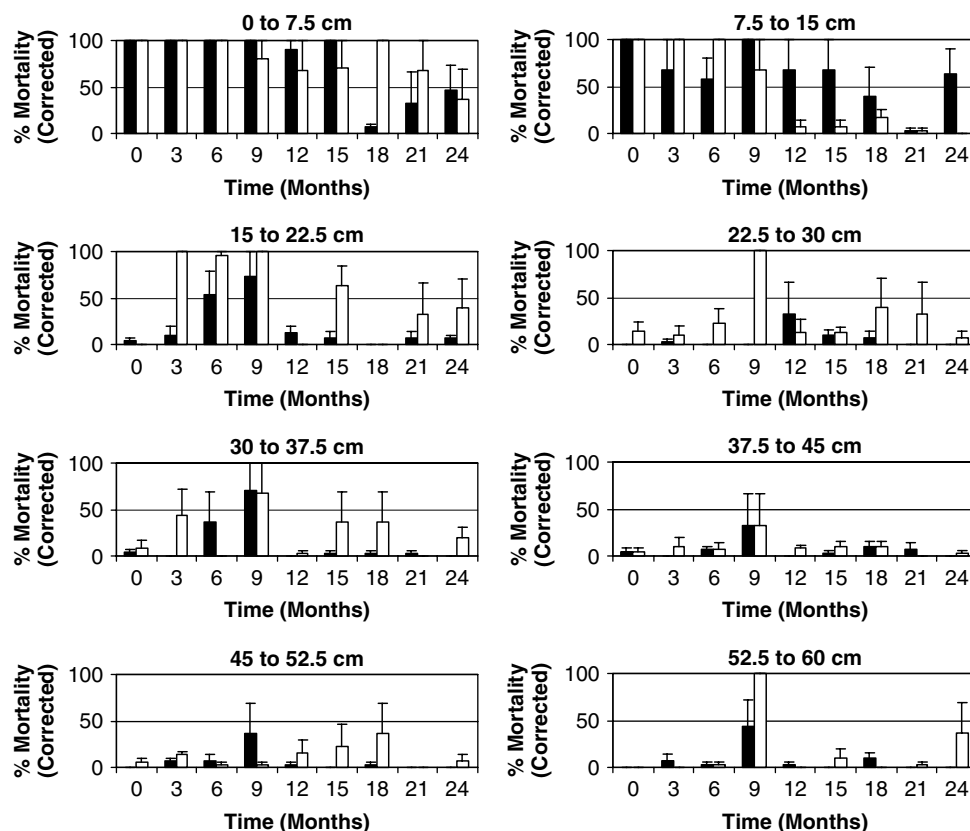


Figure 4. Percentage mortality (corrected) of termites exposed to soil from vegetated (solid bars) and non-vegetated (open bars) PVC pipes at each soil depth.

where soil from the untreated depths of non-vegetated pipes usually had higher extractable imidacloprid and were more toxic to termites than corresponding depths of vegetated pipes. However, mortality at any untreated soil depth beyond 15 months was low. It has been suggested that soil mobility may be of advantage by spreading the compound through a larger volume of soil to increase protection.¹³ A reduction in survival of termites in soil adjacent to the treated soil should have been evident if this were true. Such an effect, although observed in this study in soil immediately below the treated soil (the 15–22.5 cm depth), lasted from 3 to 12 months after treatment. Termite mortality generally was not high at other soil depths (22.5–60 cm) throughout the study. This finding is supported by a field study where imidacloprid did not decrease termite incidence at monitor stations near treated soil.⁶

4 CONCLUSIONS

Based on the results presented here, vegetation should have little effect on the longevity of an imidacloprid perimeter treatment to soil for the prevention of termite infestation. Although vegetation affected extractable imidacloprid for a period of time, this effect can be described by the influence of vegetation on soil moisture, rather than by the vegetation itself (such as translocation out of the soil into plant tissue). The effect observed is unlikely to affect the performance of the application.

It should be reinforced, however, that any gardening or landscape activities that occur in or near treated soil have the potential physically to disrupt the integrity of the treated barrier. Any soil removed from a treated area should be retained and replaced once the work is finished, or, if fresh soil is used, it should be treated at the recommended rate to restore the barrier.

Movement of imidacloprid into untreated portions of the soil was demonstrated, but is unlikely to increase the volume of soil that is toxic to termites for more than a short period of time.

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